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A COMPARATIVE STUDY ON KARYOTYPES AND CHROMOSOME BANDING PATTERN OF THREE ODOR FROGS OF *Rana*

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Abstract The chromosomes of three odor frogs, Luetuosae-group of *Rana* were analyzed by conventional, as well as C-banding and silver staining techniques. The three species here examined all had 26-chromosome karyotypes encompassing 5 large and 8 small homologous pairs. Differences among them were found in gross shapes of chromosomes and positions of secondary constrictions. Generally speaking, karyotypes of *R. andersonii* and *R. grahami* resembled each other, while the karyotype of *R. tiannanensis* differed from the former two in several respects.

Analyses of karyotypes by C-banding technique indicated that, centromeric areas of every chromosome and interstitial parts of some chromosomes of each species were heterochromatinized, and differences of distribution of heterochromatin were found among species. In early metaphase plates of *R. andersonii*, much more heterochromatinized areas were observed, and when it reached late metaphase, the numbers of heterochromatin sections in each chromosome pair reduced to a limited level, e. g. centromeres and a few interstitial parts.

The active nucleolar organizer regions (NORs) were localized in long arms of pair No. 10, in connection with secondary constrictions for *R. andersonii* and *R. grahami* as in other odor frogs previously reported, but in the long arm of pair No. 6 for *R. tiannanensis*.

The cytogenetic and taxonomic implications of the findings were discussed based on comparisons with each other, and with published literature.

Key words Amphibia, Ranidae, *Rana*, Karyotype, C-banding pattern, Ag-NORs

1 Introduction

Cytogenetic analyses among species of genus *Rana* have been carried out predominately on conventionally stained and / or using various banding techniques. To date, over 100 species of this large genus have been karyologically examined. Studies of this kind have been contributed much to the understanding of karyological evolution in the Anura (Morescalchi, 1973; Schmid, 1978; Kuramoto, 1990).

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Twelve species of *Rana* Distributed in south China, including Hainan and Taiwan form a distinct group, The "odor frogs", for which Fei *et al.*, (1990) erected a new genus *Odorrana*. Of these, several species have been karyologically examined. In the present study, karyotype, C-banding pattern and Ag-NORs of three species of this group were examined and comparisons were taken out with each other and with other related species previously reported.

2 Materials and Methods

Specimens of *Rana andersonii* (two males and three females) were collected in Tengchun and Jingdong, Yunnan; *R. grahami* (two females and one male) in Yangbi, Yunnan and Zhaotong, Yunnan; *R. tiannanensis* (one male and one female) in Lingshui, Hainan.

Metaphase chromosome spreads were prepared from bone marrow cells either in the field after Omura's (1967) method or in the laboratory by the conventional air-dry method. The animals were injected intraperitoneally with 0.1 ml of colchicine solution (0.01%) per-gram of body weight 12 to 15 hours before being sacrificed. The hypotonic treatments were made with KC1 solution (0.4%) for 30 to 45 minutes. Diploid chromosome numbers were determined by observing more than 100 metaphase plates for each species. Ten well-spread plates were photographed for each species to obtain chromosome measurements. Relative length and arm ratio were then calculated for each chromosome pair. Chromosome pairs were numbered in the order of decreasing mean relative lengths. Centromeric positions were designed based on the criteria of levan *et al.* (1964) as modified by Green *et al.* (1980).

The method for staining of constitutive heterochromatin (C-banding) was after Sumner (1972) and modified as follows. The slides were incubated for 30 minutes at room temperature in 0.1N HC1, then for 7-10 minutes at 38℃ in 5% Barium Hydroxide, and briefly washed in 0.1N HC1 and incubated in a series of 75-100% ethyl alcohol, and subsequently incubated in 2×SSC at 65℃, washed in water and finally stained with Giemsa solution (10%, pH6.8) for 10 minutes. Staining of the NORs was according to the method of Howell and Black (1980).

Because the samples of *R. andersonii* and *R. grahami* from different localities were found to resemble each other, we pooled the data together respectively.

3 Results

3.1 Karyotype

The karyotype of *R. andersonii* had 2n=26(NF=52) chromosomes, composed of five large and eight small pairs (Figure plate Ⅰ, Ⅱ). Pair Nos. 2, 7, 12, 13 were submetacentric, No. 3 was intermediate between submetacentric and metacentric, and the remaining eight pairs were metacentric (Table 1), Pair No. 10 had a secondary con-

striction on the long arm and No. 3 had another constriction on the short arm. *R. grahami* had 2 n = 26(NF = 52) chromosomes, with 5 large and 8 small homologous pairs (Figure Plate Ⅱ). Pair Nos. 2, 3, 7, 9, 12, 13 were submetacentric, and the remaining 7 pairs were metacentrics (Table 1). Only one weak secondary constriction was observed on the long arm of pair No. 10. The karyotype of *R. tiannanensis* had 2 n = 26 chromosome encompassing 5 large and 8 small pairs, but the chromosome shapes were remarkably different from the other two species. Pair Nos. 2, 7, 10, 11, 13 were submetacentric, the remaining eight pairs were metacentric. Nos. 6 and 7 had astonishing large secondary constrictions. No heteromorphic pairs were observed in either the male or the female karyotypes in three species. Therefore, three species all had 2 n = 26 (NF = 52) chromosomes comprising five large and eight small pair (see Figure Plate Ⅰ, Ⅱ), which agree, in general, with the common karyotype of Ranidae. However, as for the chromosome shapes, differences were observed among the three species.

Table 1 Relative length (RL) and arm ratio (AR) of the chromosome pairs in three species of odor frog species of *Rana* (*N* = 10; Mean ± SD)

pair	*R. andersonii*		*R. grahami*		*R. tiennanensis*	
No.	RL	AR	RL	AR	RL	AR
1	14.71 ± 0.56	1.29 ± 0.07 m	14.56 ± 1.04	1.26 ± 0.06 m	14.24 ± 1.32	1.28 ± 0.07 m
2	12.36 ± 0.59	2.60 ± 0.11 sm	12.33 ± 0.92	1.84 ± 0.07 sm	12.57 ± 1.16	2.12 ± 0.11 sm
3	12.15 ± 0.51	1.66 ± 0.04sm / m	11.04 ± 0.82	2.16 ± 0.17 sm	11.24 ± 0.96	1.34 ± 0.06 m
4	10.88 ± 0.72	1.39 ± 0.05 m	10.94 ± 0.78	1.21 ± 0.08 m	10.55 ± 0.87	1.41 ± 0.06 m
5	9.57 ± 0.61	1.37 ± 0.04 m	9.58 ± 0.87	1.38 ± 0.05 m	9.52 ± 0.72	1.37 ± 0.09 m
6	6.19 ± 0.32	1.15 ± 0.06 m	6.67 ± 0.71	1.07 ± 0.03 m	7.43 ± 0.84	1.42 ± 0.10 m
7	5.62 ± 0.38	1.94 ± 0.24 sm	5.85 ± 0.42	2.59 ± 0.14 sm	6.58 ± 0.48	1.84 ± 0.12 sm
8	5.46 ± 0.39	1.27 ± 0.11 m	5.64 ± 0.46	1.25 ± 0.09 m	5.82 ± 0.66	1.16 ± 0.09 m
9	5.23 ± 0.49	1.63 ± 0.14 m	5.37 ± 0.39	1.92 ± 0.14 sm	5.21 ± 0.54	1.23 ± 0.07 m
10	5.13 ± 0.43	1.36 ± 0.12 m	5.08 ± 0.47	1.32 ± 0.06 m	4.72 ± 0.36	2.46 ± 0.17 sm
11	4.46 ± 0.44	1.62 ± 0.13 m	4.89 ± 0.32	1.54 ± 0.12 m	4.34 ± 0.28	1.96 ± 0.15 sm
12	4.38 ± 0.39	1.86 ± 0.12 sm	4.42 ± 0.24	1.79 ± 0.07 sm	4.08 ± 0.32	1.28 ± 0.08 m
13	3.69 ± 0.21	2.02 ± 0.14 sm	3.93 ± 0.25	1.83 ± 0.10 sm	3.84 ± 0.22	2.10 ± 0.13 sm

Abreviations: m, sm, st and t represent metacentric, submetacentric, subtelocentric and telocentric chromosomes respectively

3.2 C-banding

Centromeric constitutive heterochromatin were observed in each chromosome of each species. C-banding patterns differed from species to species in interstitial areas of some chromosomes. *R. grahami* had only one interstitial C-band in long arm of No. 10, in coincidence with secondary constriction on this chromosome pair. *R. tiannanensis* had three intersitial C-bands, one in short arm of pair No. 4, the other two in pair No. 7 and 11 respectively. It was interesting to find that the heterochromatic sections were quite variable in different development stages of mitosis of *R. andersonii*. In early metaphase, as many as

3 to 8 or more C-bands were observed, while in late metaphase, we found only centromeric C-bands and one interstitial band in the long arm of No. 2. Telomeric C-band was almost absent in chromosomes of *R. grahami* and *R. tiannanensis*, but present in some chromosomes of *R. andersonii*, especially in most chromosomes of early metaphase. No heteromorphic pairs were found related to sex.

3.3 Ag-NORs

Silver-stained NORs were confirmed to be localized in the long arm of pair No. 10 for *R. andersonii* and *R. grahami*, in coincidence with secondary constrictions. Ag-NORs of *R. tiannanensis* were localized in the long arm of pair No. 6, also in coincidence with secondary constriction. No other pairs of chromosomes were found to have active Ag-NORs.

4 Discussion

The basic chromosome number of the family Ranidae is 2 n = 26 with some exceptions (King, 1990; Kuramoto, 1989, 1990; Morescalchi, 1973). All of the three species examined here, as well as other "odor frogs" examined previously. had 2 n = 26 (NF = 52) chromosomes consisting of five large and eight small pairs, as in many members of the family. To date, seven out of twelve species of odor frogs have been karyologically examined, Differences were observed in chromosome shapes, numbers and positions of secondary constrictions and C-banding patterns (Chen *et al.*, 1983; Li *et al.*, 1982; Wang *et al.*, 1983; Wu *et al.*, 1989; Xu *et al.*, 1990; Liu *et al.*, 1993; Wei *et al.*, 1993). In the present study , the three species differed both in conventional and banded karyotype. When compared, we found that *R. andersonii* and *R. grahami* were similar in the shapes of most chromosomes, but differed in secondary constrictions. *R. andersonii* has a secondary constriction on the short arm of No. 3, which is absent in *R. grahami*. The karyotype of *R. tiannanensis* differs remarkably from the former two both in chromosome shapes and secondary constrictions. It has two astonishingly large secondary constrictions on the long arms of pair Nos. 6 and 7. This character also differentiates *R. tiannanensis* from all other odor frogs.

Centromeric C-bands were observed to be commonly present in each chromosome of each species, but with regard to the interstitial and telomeric bands, differences were clearly observed. *R. grahami* had only one interstitial C-band in long arm of No. 10. *R. tiannanensis* had three interstitial C-bands. The situation in *R. andersonii* is unique. In the early stage of metaphase, as many as 8 C-bands were observed, but when in late metaphase, the number of C-bands was reduced to a limited number. This is a interesting finding, but very difficult to understand. Because the mechanisms of CBG technique are not yet clear, we don't know the definite factor(s) responsible for the difference of C-band beterochromatin between early and late metaphase chromosomes. This phenomenon might have been observed by many

researchers, but omitted by them. For example, Schmid (1978) observed high numbers of C-bands in chromosomes of *R. esculenta*, but we note that he might have counted the numbers based on an early metaphase plate. A problem arises with reliability when C-banding patterns of related species are compared. To prevent the misunderstanding, we must be sure that the stages of metaphase plates to be compared should be approximately same.

In most of the *Rana* species studied using the silver-staining method, the Ag-NORs lie within the secondary constriction in the long arm of chromosome pair No. 10. This is the same situation for odor frogs previously studied. In the present study, as in many other *Rana*, Ag-NORs of *R. andersonii* and *R. grahami* were localized on the long arms of No. 10. but Ag-NORs of *R. tiannanensis* were in the long arm of No. 6, which differentiated it from all other odor frogs. Some species of the odor frog group were examined using samples from different geographical populations, because differences were observed between local populations. Karyotype, C-banding and Ag-NORs of *R. andersonii* from Guizhou were reported by Wei *et al.* (1993). When compared with the Yunnan population presented in this work, it is interesting to note that pair No. 3 of specimens from Yunnan had a secondary constriction in the short arm, that was absent in speimens from Guizhou. A strong C-band was observed present in long arm of No. 2 in Yunnan specimens, but absent in Guizhou specimens. Guizhou samples revealed to have a weak interstitial C-band in the short arm of No. 3, but samples from Yunnan have not. Those differences can be explained as chromosome polymorphisms in populations and may be caused by inversions and / or translocations.

Wei *et al.* (1993) postulated that *R. margaratae* could be the most original and *R. kuangwunesis* the most specialized in the light of cytogenetics. In this study, we found that the karyotype of *R. tiannanensis* was different from all other odor frogs in chromosome shapes, positions of secondary constrictions, as well as C-banding pattern and locations of Ag-NORs. This species is distributed only in tropical areas of Yunnan and Hainan Island, but most other species of the group are distributed on the Yunnan and Guizhou Plateau, especially in Guizhou (Wei *et al.*, 1993). Therefore, *R. tiannanensis* could have been separated from other species earlier, and evolved independently.

图 版 说 明

图版 I

A-C: karyotype (A), Ag-NORs(B) and C-banding (C) of *R. andersonii.*

D-F: C-banded plates of *R. andersonii* showing reduction in number of C-bands from early to late metaphase.

图版 II

G–I: Karyotype(G), C–banding(H) and Ag–NORs(I) of *R. grahami*.

J–L: karyotype(J), C–banding(K) and Ag–NORs(L) of *R. tiannanensis*.

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臭蛙群三种蛙类核型及染色体带型的比较

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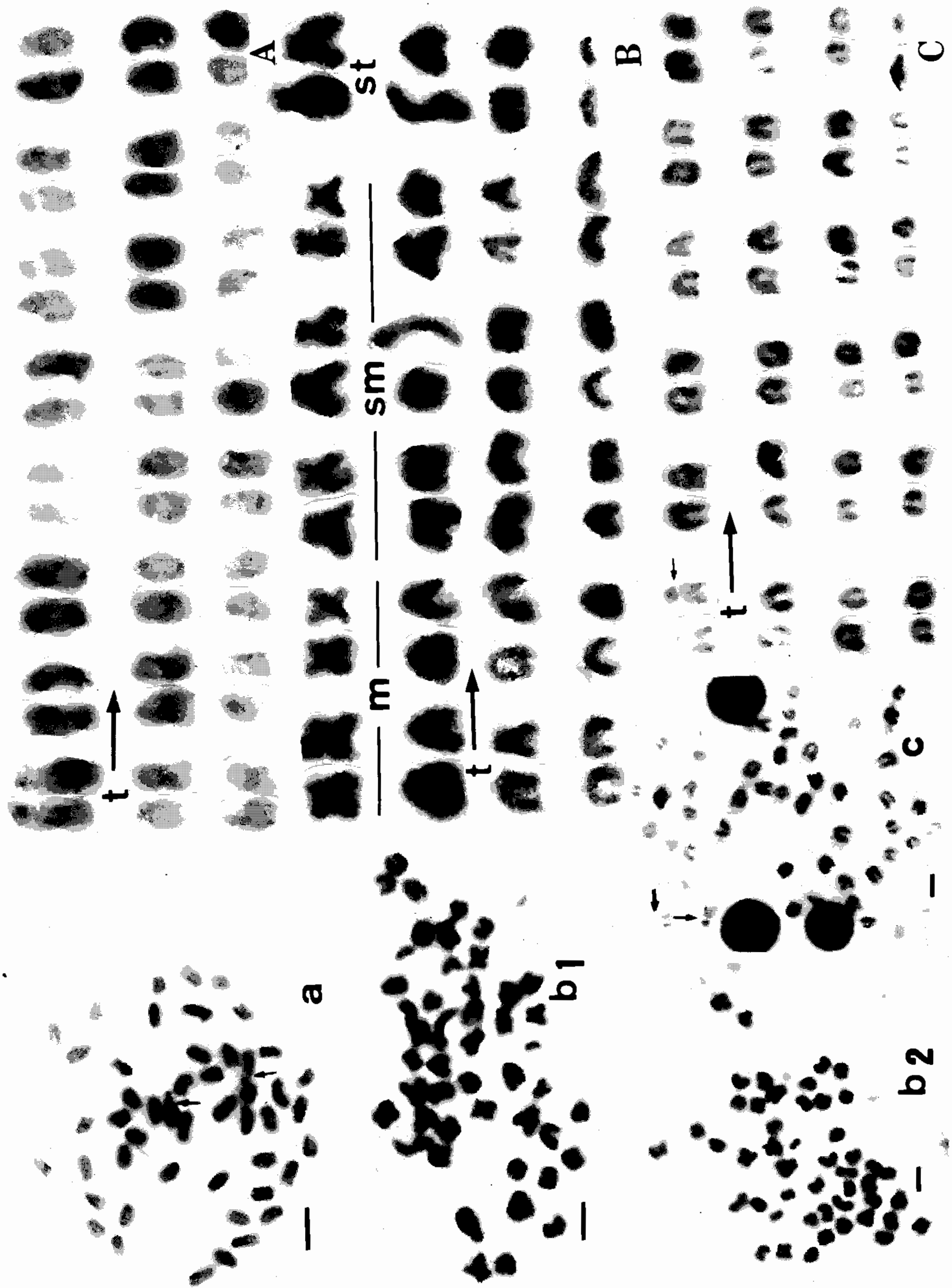
摘要　本文比较了3种臭蛙，即云南臭蛙(*R. andersonii*)、无指盘臭蛙(*R. grahami*)和滇南臭蛙(*R. tiennanensis*)的核型、C-带和银染核仁组织者区(Ag-NORs)。结果表明，3种臭蛙的核型均为2n=26(NF=52)，由5对大型染色体和8对小型染色体组成，与蛙科大多数种类的核型相似，但是染色体形态和次缢痕的位置和数目有差异。其中，云南臭蛙和无指盘臭蛙的核型相似程度比较大，与其他已报道的臭蛙类的核型相差不大，而滇南臭蛙的核型比较特殊，与其他臭蛙类的核型存在显著差异。

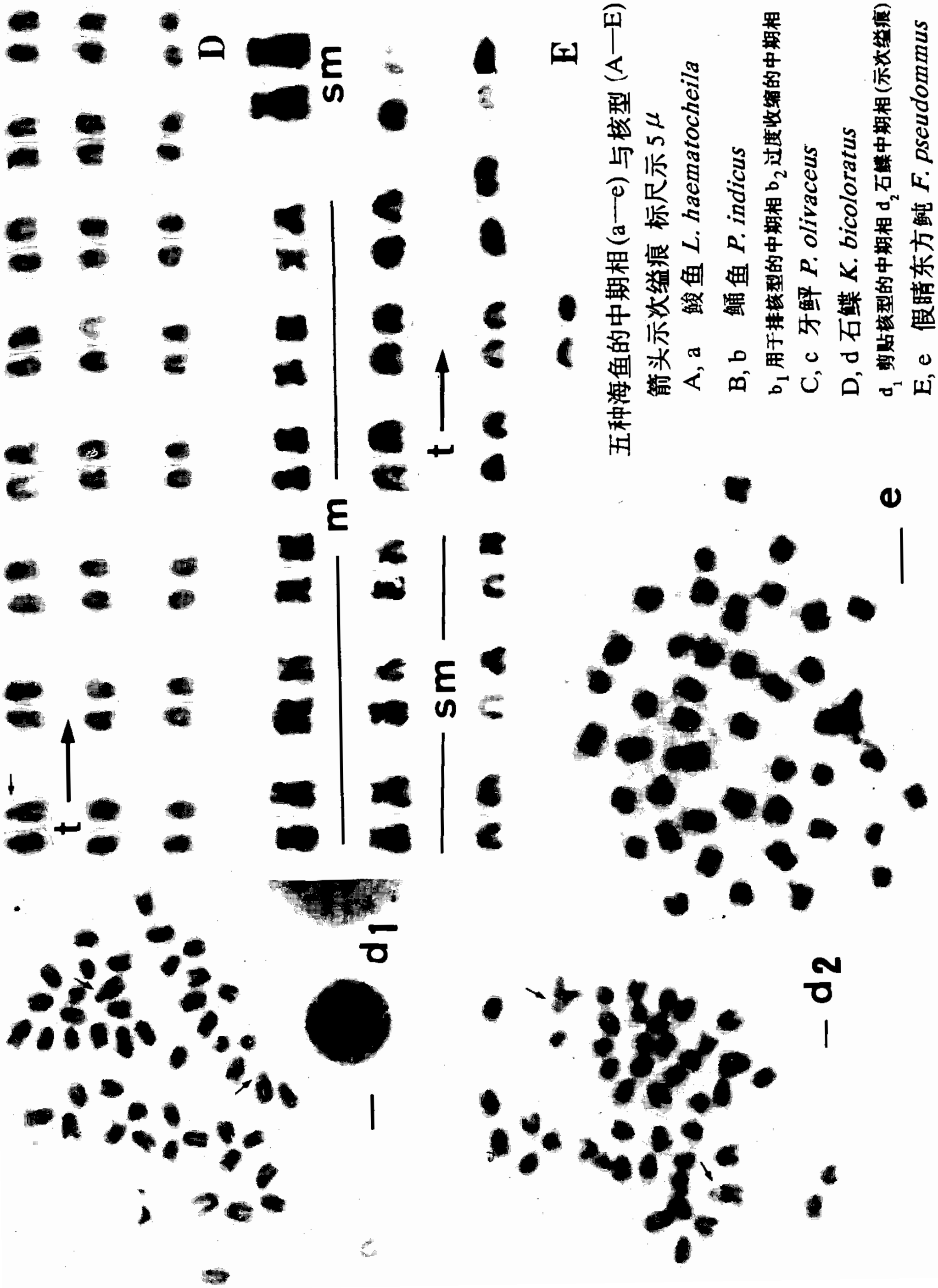
C-带技术显示的结果表明，3种臭蛙所有染色体的着丝点都有比较显著的C-带，而居间区C-带和端点C-带却存在明显的差异。3种中，滇南臭蛙的C-带带型比较特殊。有趣的是在研究中还发现，云南臭蛙的早中期细胞(染色体很长)染色体上发现了很多结构异染色质区，除着丝点C-带外，几乎所有的染色体上都有居间区或端点C-带，而在晚中期细胞中所观察到的C-带数目却较少，除着丝点外，只在少数染色体上发现了C-带。

云南臭蛙和无指盘臭蛙的Ag-NORs位于第10对染色体的长臂上，与该染色体上的次缢痕位置相对应，而滇南臭蛙的Ag-NORs位于第6对染色体的长臂上。

综上所述，云南臭蛙与无指盘臭蛙的核型及带型较相似，与其他臭蛙相比也有不少共同之处，而滇南臭蛙的核型和带型在臭蛙中最特殊。

关键词　蛙科，蛙属，核型，C-带，Ag-NORs


A
st
sm
m
t
B
C
a
b1
b2
c



五种海鱼的中期相(a—e)与核型(A—E)
箭头示次缢痕　标尺示5μ
A, a　鮻鱼 *L. haematocheila*
B, b　鲬鱼 *P. indicus*
b_1 用于排核型的中期相 b_2 过度收缩的中期相
C, c　牙鲆 *P. olivaceus*
D, d　石鲽 *K. bicoloratus*
d_1 剪贴核型的中期相 d_2 石鲽中期相(示次缢痕)
E, e　假睛东方鲀 *F. pseudommus*